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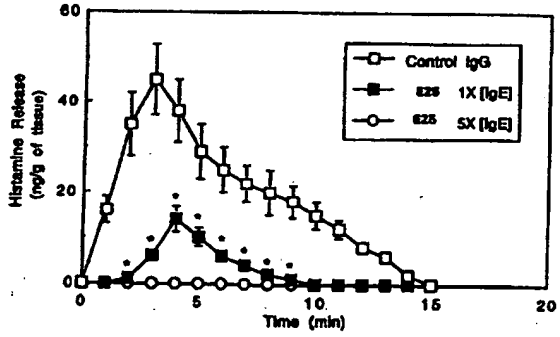
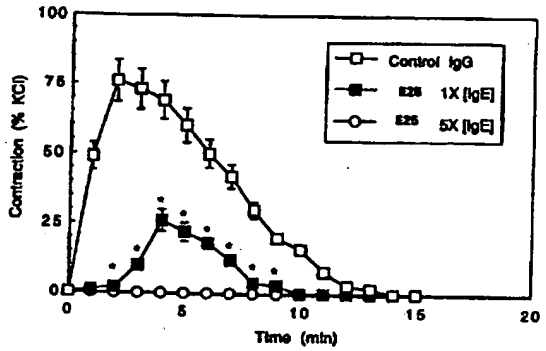
(57) Abstract

Methods of treatment of interstitial cystitis with IgE antagonists, including anti-IgE antibodies, IgE variants, peptide antagonists, peptidomimetics and other small molecules, are described.





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METHODS FOR TREATMENT OF INTERSTITIAL CYSTITIS

FIELD OF THE INVENTION

This invention relates to methods of treatment of interstitial cystitis with IgE antagonists, including anti-IgE antibodies.

BACKGROUND OF THE INVENTION

Interstitial cystitis (IC) is a condition of the bladder characterized by urinary frequency, urgency, and suprapubic pain and pressure. Typically, the disease is diagnosed on the basis of cytoscopic appearance or pathological findings.

Immunological responses are associated with numerous urological diseases such as IC, bladder cancer, and bladder infection (Van de Merwe et al. J. Rheumatol. 20: 962, 1993). These responses include an increased number of macrophages, activated lymphocytes, and vascular endothelial cells expressing HLA class II molecules within the submucosa (Christmas et al. Clin. Exp. Immunol. 87:450, 1992), increased expression of HLA-DR in urothelial cells (Liebert et al. J. Urol. 149:470, 1993), and alteration of lymphocyte sub-populations (CD4:CD8 ratio) in the bladder tissue of IC patients (MacDermott J. Urol. 145:274, 1991). Some investigators have suggested that the immune responses are likely to be secondary phenomena associated with inflammatory damage to the bladder (Anderson et al. Br. J. Urol. 63:58, 1989). However, autoimmune or allergic disorders have been suggested among the potential causes of non-infectious cystitis, including IC (Hand et al. J. Urol. 61:291, 1949; Messing et al. Campbell's Urology, 6th ed., P.C. Walsh et al. eds., Philadelphia, W.B. Saunders Co. pp 982-1005, 1991; Holm-Bentzen et al. J. Urol. 138: 500, 1987). Experimentally-induced autoimmune cystitis has many features similar to those observed in clinical IC (Bullock et al. J. Urol. 148:1951, 1992). Furthermore, reports on IC indicate that at least 50% of IC patients have some form of allergy (Koziol. Urol. Clin. of N. Amer. 21:7, 1994). Thus, an immunological etiology involving mast cells appears to occur in at least a subset of IC patients.

Bjorling et al. (J. Urol. 152:1603, 1994) describe an experimental model for non-infectious cystitis in which bladder tissue from humans, guinea pigs, or Rhesus monkeys is passively sensitized in vitro by incubation with serum containing antigen-specific immunoglobulin. In this model, subsequent antigen challenge stimulates contraction and histamine release in the sensitized tissue.

Haak-Frenscho et al. ("Human FCεRI-IgG and Humanized anti-IgE monoclonal Antibody Mae11 Block Passive Sensitization of Human and Rhesus Monkey Bladder", presented at 1995 Interstitial Cystitis Symposium, January 9-11), which is not prior art to this invention, disclose both FCεRI-IgG immunoadhesin and humanized anti-IgE antibody abolished IgE-induced contraction and histamine release in an in vitro IC model system.

The concept of using anti-IgE antibodies as a treatment for allergy has been widely disclosed in the scientific literature. A few representative examples are as follows. Baniyash and Eshhar (European Journal of Immunology 14:799-807 (1984)) demonstrated that an anti-IgE monoclonal antibody could specifically block passive cutaneous anaphylaxis reaction when injected intradermally before challenging with the antigen; U.S. 4,714,759 discloses a product and process for treating allergy, using an antibody specific for IgE; and Rup and Kahn (International Archives Allergy and Applied Immunology, 89:387-393 (1989) discuss the prevention of the development of allergic responses with monoclonal antibodies which block mast cell-IgE sensitization.

Anti-IgE antibodies which block the binding of IgE to its receptor on basophils and which fail to bind to IgE bound to the receptor, thereby avoiding histamine release are disclosed, for example, by Rup and Kahn (supra), by Baniyash et al. (Molecular Immunology 25:705-711, 1988), and by Hook et al. (Federation of American Societies for Experimental Biology, 71st Annual Meeting, Abstract #6008, 1987).

Antagonists of IgE in the form of receptors, anti-IgE antibodies, binding factors, or fragments thereof have been disclosed in the art. For example, U.S. 4,962,035 discloses DNA encoding the alpha-subunit of the mast cell IgE receptor or an IgE binding fragment thereof. Hook *et al.* (*Federation Proceedings* Vol. 40, No. 3, Abstract #4177) disclose monoclonal antibodies, of which one type is anti-idiotypic, a second type binds to common IgE determinants, and a third type is directed towards determinants hidden when IgE is on the basophil surface.

U.S. 4,940,782 discloses monoclonal antibodies which react with free IgE and thereby inhibit IgE binding to mast cells, and react with IgE when it is bound to the B-cell FcE receptor, but do not bind with IgE when it is bound to the mast cell FcE receptor, nor block the binding of IgE to the B-cell receptor.

U.S. 4,946,788 discloses a purified IgE binding factor and fragments thereof, and monoclonal antibodies which react with IgE binding factor and lymphocyte cellular receptors for IgE, and derivatives thereof.

U.S. 5,091,313 discloses antigenic epitopes associated with the extracellular segment of the domain which anchors immunoglobulins to the B cell membrane. The epitopes recognized are present on IgE-bearing B cells but not basophils or in the secreted, soluble form of IgE. U.S. 5,252,467 discloses a method for producing antibodies specific for such antigenic epitopes. U.S. 5,231,026 discloses DNA encoding murine-human antibodies specific for such antigenic epitopes.

U.S. 4,714,759 discloses an immunotoxin in the form of an antibody or an antibody fragment coupled to a toxin to treat allergy.

Presta et al. (J. Immunol. 151:2623-2632 (1993)) disclose a humanized anti-IgE antibody that prevents the binding of free IgE to FcεRI but does not bind to FcεRI-bound IgE. Copending WO93/04173 discloses polypeptides which bind differentially to the high- and low-affinity IgE receptors. Copending WO93/04173 discloses IgE antagonists comprising one or more of the FcεRI receptor-binding determinant sites of human IgE.

U.S. 5,428,133 discloses anti-IgE antibodies as a therapy for allergy, especially antibodies which bind to IgE on B cells, but not IgE on basophils. U.S. 5,422,258 discloses a method for making such antibodies.

Haak-Frenscho et al. (J. Immunol. 151:351-358, 1993) disclose an FCεRI-IgG immunoadhesin which is a fusion of the extracellular portion of the human α-chain of FCεRI, which contains the high affinity binding site for IgE, with a truncated human IgG1 heavy chain constant region.

SUMMARY OF THE INVENTION

One embodiment of the invention is a method of treatment of interstitial cystitis in a patient comprising administering a therapeutic dose of an IgE antagonist to the patient.

Another embodiment of the invention is a method of reducing histamine release from mast cells in the bladder tissue of a patient with interstitial cystitis comprising administering a therapeutic dose of an IgE antagonist to the patient.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graph depicting representative concentration-dependent contraction of human bladder tissue segments sensitized by incubation with serum from a ragweed allergic patient and induced by cumulative addition of ragweed antigen (antigen E or AgE). The change in the symbols represents cumulative addition of increasing concentrations of AgE (0.01, 0.1, 1.0, and 3.0 μg/ml) or KCl (200 mM). The maximal effect was obtained with 1.0 μg/ml AgE. The highest concentration tested, 3.0 μg/ml, reduced contraction in the tissue. The graph represents tissue contractability and viability results obtained with eight different bladders.

Figure 2 is a graph depicting concentration-dependent contraction of sensitized human bladder tissue segments induced by addition of a single concentration of AgE ((0.01, 0.1, 1.0, and 3.0 μg/ml). Responses are represented as a percentage of the maximum obtained in response to KCl (200 mM) added at the end of the experiment. The graph represents results obtained with eight different bladders.

Figure 3 (A-D) represents polygraph tracings representative of tissues isolated from eight different bladders. Adjacent segments of each bladder were passively sensitized, suspended in vitro, and challenged with AgE (1 μg/ml) at 10 min (first arrow). Contractions are expressed as grams of tension. Segment A was a non-sensitive negative control; segment B was a sensitized positive control incubated with control IgG; segment C was incubated with anti-IgE monoclonal antibody E25 (1:1 concentration of E25 to IgE) during sensitization; segment D was incubated with E25 (5:1 concentration of E25 to IgE) during sensitization. KCl (200 mM) was added at the end of the experiment (second arrow).

Figure 4 (A and B) represents IgE antagonist blocking of histamine release (A) and tissue contraction (B) in a concentration-dependent manner in sensitized human bladder tissue. Human bladder strips were incubated with a 1:10 dilution of human ragweed serum for 20 hr. in the presence of equimolar, 5-fold, or 10-fold excess concentrations of E25 or a 10-fold concentration of control IgG in excess of the serum IgE concentration. IgG had no detectable effect on either histamine release (A) or tissue contraction (B). In contrast, E25 blocked both histamine release and tissue contraction in response to IgE challenge in a concentration-dependent manner. Data are presented as the mean ± SEM of eight separate experiments. Peak histamine release occurred at 3 min. and correlated with the onset of contraction. In all experiments, adjacent bladder strips incubated with physiologic salt solution (PSS) only (nonsensitized) for 20 hr did not respond to AgE challenge. Asterisks indicate time points at which a significant difference was observed ($p<0.05$) compared to control IgG responses.

Figure 5 (A and B) represents IgE antagonist blocking of histamine release (A) and tissue contraction (B) in a concentration-dependent manner in sensitized Rhesus monkey bladder tissue. Rhesus monkey bladder strips were incubated with a 1:10 dilution of human ragweed serum for 20 hr in the presence of equimolar, 5-fold, or 10-fold excess concentrations of E25 or a 10-fold concentration of control IgG in excess of the serum IgE concentration. IgG had no detectable effect on either histamine release (A) or tissue contraction (B). In contrast, E25 blocked both histamine release and tissue contraction in response to AgE challenge in a concentration-dependent manner. Data are presented as the mean ± SEM of 14 separate experiments. Peak histamine release occurred at 3 min and correlated with the onset of contraction. In all experiments, adjacent bladder strips incubated with PSS only (nonsensitized) for 20 hr did not respond to AgE challenge. Asterisks indicate time points at which a significant difference was observed ($p<0.05$) compared to control IgG responses.

Figure 6 is a graph depicting degranulation and tissue contraction in passively sensitized human (full bar) or monkey (empty bar) bladder tissues. Bladder segments were passively sensitized with ragweed serum, washed with PSS and challenged with 1 μg/ml E25. Positive control bladders challenged with AgE exhibited vigorous bladder contraction (percent of KCl maximum and histamine release (ng/g of tissue). In contrast, challenge with E25 did not result in any detectable histamine release or tissue contraction. Non-sensitized bladders segments (negative control) challenged with AgE did not respond. Results are expressed as the mean responses of tissue from five human bladders and 10 monkey bladders. Asterisks and SEM are not shown for sake of clarity. A significant difference ($p<0.05$) was found between AgE and E25 challenge of sensitized tissues.

DETAILED DESCRIPTION OF THE INVENTION

A. DEFINITIONS

The term "interstitial cystitis' as used herein is intended to refer to a bladder condition characterized by urinary frequency, urgency, and suprapubic pain and pressure.

The term "IgE antagonist" as used herein refers to a substance which inhibits the biological activity of IgE. Such antagonists include but are not limited to anti-IgE antibodies, immunoadhesins, IgE receptors, anti-IgE receptor antibodies, variants of IgE antibodies, ligands for the IgE receptors, and fragments thereof. Antibody antagonists can be of the IgA, IgD, IgG, IgE, or IgM class. Bispecific antibodies can also be used. Variant IgE antibodies typically have amino acid substitutions or deletions at one or more amino acid residues. Ligands for IgE receptors include but are not limited to IgE and anti-receptor antibodies, and fragments thereof capable of binding to the receptors, including amino acid substitution and deletion variants, and cyclized variants.

In general, in some embodiments of the invention, IgE antagonists act by blocking the binding of IgE to its receptors on B cells, mast cells, or basophils, either by blocking the binding site on the IgE molecule or blocking its receptors. Additionally, in some embodiments of the invention, IgE antagonists act by binding soluble IgE and thereby removing it from circulation. The IgE antagonists of the invention can also act by binding to IgE on B cells, thereby eliminating clonal populations of B cells. The IgE antagonists of the instant invention can also act by inhibiting IgE production. Preferably, the IgE antagonists of the instant invention do not result in histamine release from mast cells or basophils.

The term "therapeutic amount" as used herein denotes an amount that prevents or ameliorates symptoms of a disorder or responsive pathologic physiological condition.

"Polypeptide" as used herein refers generally to peptides and proteins having at least about two amino acids.

The term "free IgE" as used herein refers to IgE not complexed to a binding partner, such an anti-IgE antibody. The term "total IgE" as used herein refers to the measurement of free IgE and IgE complexed to a binding partner, such as an anti-IgE antibody. The term "baseline IgE" as used herein refers to the level of free IgE in a patient's serum before treatment with an IgE antagonist.

The term "polyol" as used herein denotes a hydrocarbon including at least two hydroxyls bonded to carbon atoms, such as polyethers (e.g. polyethylene glycol), trehalose, and sugar alcohols (such as mannitol).

The term "polyether" as used herein denotes a hydrocarbon containing at least three ether bonds. Polyethers can include other functional groups. Polyethers useful for practicing the invention include polyethylene glycol (PEG).

B. GENERAL METHODS

Polyclonal antibodies to IgE generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of IgE and an adjuvant. It can be useful to conjugate IgE or a fragment containing the target amino acid sequence from the Fc region of IgE to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N = C = NR$, where R and R^1 are different alkyl groups.

Animals ordinarily are immunized against the cells or immunogenic conjugates or derivatives by combining 1 mg or 1 μg of IgE with Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of conjugate in Freund's incomplete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later, animals are bled and the serum is assayed for anti-IgE titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with a conjugate of the same IgE, but conjugated to a different protein and/or through a different cross-linking agent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum can be used to enhance the immune response.

Monoclonal antibodies are prepared by recovering spleen cells from immunized animals and immortalizing the cells in conventional fashion, e.g. by fusion with myeloma cells or by Epstein-Barr (EB)-virus transformation and screening for clones expressing the desired antibody. The hybridoma technique described originally by Koehler and Milstein, Eur. J. Immunol., 6: 511 (1976) and also described by Hammerling *et al.*, in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens.

The hybrid cell lines can be maintained *in vitro* in cell culture media. The cell lines producing the antibodies can be selected and/or maintained in a composition comprising the continuous cell line in hypoxanthine-aminopterin thymidine (HAT) medium. In fact, once the hybridoma cell line is established, it can be maintained on a variety of nutritionally adequate media. Moreover, the hybrid cell lines can be stored and preserved in any number of conventional ways, including freezing and storage under liquid nitrogen. Frozen cell lines can be revived and cultured indefinitely with resumed synthesis and secretion of monoclonal antibody.

The secreted antibody is recovered from tissue culture supernatant by conventional methods such as precipitation, ion-exchange chromatography, affinity chromatography, or the like. The antibodies described herein are also recovered from hybridoma cell cultures by conventional methods for purification of IgG or IgM, as the case may be, that heretofore have been used to purify these immunoglobulins from pooled plasma, e.g., ethanol or polyethylene glycol precipitation procedures. The purified antibodies are sterile-filtered.

While routinely mouse monoclonal antibodies are used, the invention is not so limited; in fact, human antibodies can be used. Such antibodies can be obtained, for example, by using human hybridomas (Cote et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985)). In fact, according to the invention,

techniques developed for the production of chimeric antibodies (Cabilly et al., U.S. 4,816,567, Morrison et al., Proc. Natl. Acad. Sci. 81: 6851 (1984); Boulianne et al., Nature 312: 643-646 (1984); Neuberger et al., Nature, 312: 604 (1984); Neuberger et al., Nature 314: 268-270 (1985); Takeda et al., Nature 314: 452 (1985); EP 184,187; EP 171,496; EP 173,494; PCT WO 86/01533; Shaw et al., J. Nat. Canc. Inst. 80: 1553-1559 (1988); Morrison, Science 229: 1202-1207 (1985); Oi et al., BioTechniques, 4: 214 (1986)) by coupling an animal antigen-binding variable domain to a human constant domain can be used; such antibodies are within the scope of this invention. The term "chimeric" antibody is used herein to describe a polypeptide comprising at least the antigen binding portion of an antibody molecule linked to at least part of another protein (typically an immunoglobulin constant domain).

In one embodiment, such chimeric antibodies contain about one third rodent (or other non-human species) sequence and thus are capable of eliciting a significant anti-globulin response in humans. For example, in the case of the murine anti-CD3 antibody OKT3, much of the resulting anti-globulin response is directed against the variable region rather than the constant region (Jaffers et al., Transplantation 41: 572-578 (1986)).

Humanized antibodies are used to reduce or eliminate any anti-globulin immune response in humans. In practice, humanized antibodies are typically human antibodies in which some amino acid residues from the complementarity determining regions (CDRs), the hypervariable regions in the variable domains which are directly involved with formation of the antigen-binding site, and possibly some amino acids from the framework regions (FRs), the regions of sequence that are somewhat conserved within the variable domains, are substituted by residues from analogous sites in rodent antibodies. The construction of humanized antibodies is described in Riechmann et al., Nature 332: 323-327 (1988), Queen et al., Proc. Natl. Acad. Sci. USA 86: 10029-10033 (1989), Co et al., Proc. Natl. Acad. Sci. USA 88: 2869-2873 (1991), Gorman et al., Proc. Natl. Acad. Sci. 88: 4181-4185 (1991), Daugherty et al., Nucleic Acids Res. 19: 2471-2476 (1991), Brown et al., Proc. Natl. Acad. Sci. USA 88: 2663-2667 (1991), Junghans et al., Cancer Res. 50: 1495-1502 (1990), Fendly et al., Cancer Res. 50: 1550-1558 (1990) and in PCT applications WO 89/06692 and WO 92/22653.

In some cases, substituting CDRs from rodent antibodies for the human CDRs in human frameworks is sufficient to transfer high antigen binding affinity (Jones et al., Nature 321: 522-525 (1986); Verhoeyen et al., Science 239: 1534-1536 (1988)) whereas in other cases it is necessary to additionally replace one (Riechmann et al., supra) or several (Queen et al., supra) FR residues. See also Co et al., supra.

The invention also encompasses the use of human antibodies produced in transgenic animals. In this system, DNA encoding the antibody of interest is isolated and stably incorporated into the germ line of an animal host. The antibody is produced by the animal and harvested from the animal's blood or other body fluid. Alternatively, a cell line that expresses the desired antibody can be isolated from the animal host and used to produce the antibody in vitro, and the antibody can be harvested from the cell culture by standard methods.

Anti-IgE antibody fragments can also be used in the methods of the invention. Any fragment of an anti-IgE antibody capable of blocking or disrupting IgE interaction with its receptor is suitable for use herein.

Suitable anti-IgE antibody fragments can be obtained by screening combinatorial variable domain libraries for DNA capable of expressing the desired antibody fragments. These techniques for creating recombinant DNA versions of the antigen-binding regions of antibody molecules which bypass the generation of monoclonal antibodies, are encompassed within the practice of this invention. One typically extracts antibody-

specific messenger RNA molecules from immune system cells taken from an immunized animal, transcribes these into complementary DNA (cDNA), and clones the cDNA into a bacterial expression system. "Phage display" libraries are an example of such techniques. One can rapidly generate and screen great numbers of candidates for those that bind the antigen of interest. Such IgE-binding molecules are specifically encompassed within the term "antibody" as it is defined, discussed, and claimed herein.

In a further embodiment of the invention, soluble IgE receptor can be used as the IgE antagonist. Soluble receptors suitable for use herein include, for example, molecules comprising the IgE binding site in the extracellular domain (exodomain) of the FcεRI α chain. The α chain of FcεRI can be genetically modified such that the exodomain is secreted as a soluble protein in a recombinant expression system according to the method of Blank et al., J. Biol. Chem., 266: 2639-2646 (1991) or Qu et al., J. Exp. Med., 167: 1195.

The invention also encompasses the use of IgE-binding peptides in addition to anti-IgE antibodies and soluble receptor. Any IgE-binding peptide capable of disrupting or blocking the interaction between IgE and its receptors is suitable for use herein.

In addition to IgE antagonists which interfere with IgE/receptor interaction by binding to IgE, such as anti-IgE antibodies, fragments thereof, soluble IgE receptor and other IgE-binding peptides described above, the invention encompasses the use of IgE antagonists which disrupt IgE/receptor interaction by competing with IgE for binding to its receptor, thereby lowering the available IgE receptor.

IgE variants are an example of a receptor-binding competitor that is suitable for use in the methods of the invention. IgE variants are forms of IgE possessing an alteration, such as an amino acid substitution or substitutions and/or an amino acid deletion or deletions, wherein the altered IgE molecule is capable of competing with IgE for binding to its receptors.

Fragments of IgE variants are also suitable for use herein. Any fragment of an IgE variant capable of competing with IgE for binding to its receptors can be used in the methods of the invention.

The invention also encompasses the use of IgE receptor-binding peptides in addition to IgE variants and fragments thereof. Any IgE receptor-binding peptide capable of disrupting or blocking the interaction between IgE and its receptors is suitable for use herein.

The amount of IgE antagonist delivered to the patient to be used in therapy will be formulated and dosages established in a fashion consistent with good medical practice taking into account the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Similarly, the dose of the IgE antagonist administered will be dependent upon the properties of the IgE antagonist employed, e.g. its binding activity and in vivo plasma half-life, the concentration of the IgE antagonist in the formulation, the administration route, the site and rate of dosage, the clinical tolerance of the patient involved, the pathological condition afflicting the patient and the like, as is well within the skill of the physician.

Typically IgE antagonists are administered by intramuscular, intravenous, intrabronchial, intraperitoneal, intravesical, subcutaneous or other suitable routes. The antagonists can be administered before and/or after the onset of symptoms. In general, a "loading" dose of an IgE antagonist is useful to obtain a rapid and sustained decrease in free IgE. A loading dose is typically a first dose of IgE antagonist that is greater than a subsequent or "maintenance" dose of IgE antagonist. However, patients can be loaded in other ways. For

example, patients can be loaded by administering a dose of antagonist that is greater than or equal to the same mg/kg amount as the maintenance dose, but increasing the frequency of administration in a "loading regimen". Thus, for example, if the maintenance dose is 1 mg/kg biweekly, the patient can be loaded by administering 1 mg/kg weekly for two or more weeks in a row, then administering the maintenance dose of 1 mg/kg biweekly. Furthermore, patients can be loaded during a course of treatment with a maintenance dose of IgE antagonist by administering larger or more frequent doses than the maintenance dose. The term "loading dose" is intended as used herein to include such single loading doses, multiple loading doses, loading regimens, and combinations thereof.

A sustained decrease in free IgE can be obtained by administration of a maintenance dose of the antagonist. Maintenance doses are delivered with a frequency of about every day to about every 90 days, more preferably weekly to biweekly, depending on the severity of the patient's symptoms, the concentration and in vivo properties of antagonist delivered, and the formulation of the antagonist. For example, slow release formulations can allow less frequent administration. Maintenance doses can be adjusted upwards or downwards over time, depending on the response of the patient.

Thus, for example, in one embodiment of the invention, the dose of IgE antagonist is sufficient to reduce free IgE in the patient's serum to less than about 40 ng/ml.

In a further dosing strategy, about 0.05 to 10 mg/kg, more preferably about 0.1 to 1 mg/kg, most preferably about 0.5 mg/kg IgE antagonist can be administered on a weekly basis to a patient having about 40-200 IU/ml baseline IgE. In another dosing strategy for individuals with higher baseline IgE, patients are preferably "loaded" with about 1 to about 10 mg/kg, more preferably about 1 to about 5 mg/kg, most preferably about 2 mg/kg, IgE antagonist, followed by weekly or biweekly administration of about 0.1 to about 10 mg/kg, most preferably about 1 mg/kg.

In a further dosing strategy, a maintenance dose of IgE antagonist averaging about 0.0005 to 0.05 mg/kg/week for every IU/ml baseline IgE, more preferably 0.001 to about 0.01 mg/kg/week for every IU/ml baseline IgE is used. This maintenance regimen can follow an initial loading dose of about 1 to 10 mg/kg, more preferably about 1 to 5 mg/kg IgE antagonist.

In a further embodiment of the invention, sufficient IgE antagonist is provided through the maintenance dose, and, optionally, the loading dose, to achieve about a 1 to 20 fold, preferably about 3 to 5 fold, most preferably about a 5 fold greater serum concentration than total serum IgE concentration in the patient.

IgE levels are typically assayed by standard ELISA techniques well known in the art. Total serum IgE can be measured by commercially available assays, such as Abbott Laboratories' Total IgE assay. Free IgE, e.g., IgE not bound to antibody can be measured by a capture type assay in which, for example, IgE receptor is bound to a solid support. IgE complexed to an anti-IgE antibody which binds at or near the site on IgE which binds to the receptor will be blocked from binding the receptor, and thus only free or unbound IgE can react with the receptor bound to the solid support in this assay. An anti-IgE antibody which recognizes IgE even when the IgE is bound to its receptor can be used to detect the IgE captured by the receptor on the solid support. This anti-IgE antibody can be labeled with any of a variety of reporter systems, such as alkaline phosphatase, etc.

It is envisioned that injections (intravenous, intramuscular or subcutaneous) will be the primary route for therapeutic administration of the IgE antagonist of this invention, although delivery through catheter or other

surgical tubing is also used. Alternative routes include suspensions, tablets, capsules and the like for oral administration, commercially available nebulizers for liquid formulations, and inhalation of lyophilized or aerosolized microcapsules, and suppositories for rectal or vaginal administration. Liquid formulations can be utilized after reconstitution from powder formulations.

Additional pharmaceutical methods may be employed to control the duration of action of the antagonists of this invention. The antagonists also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Osol, A., ed., 1980).

In general, the formulations of the subject invention can contain other components in amounts not detracting from the preparation of stable forms and in amounts suitable for effective, safe pharmaceutical administration. For example, other pharmaceutically acceptable excipients well known to those skilled in the art can form a part of the subject compositions. These include, for example, salts, various bulking agents, additional buffering agents, chelating agents, antioxidants, cosolvents and the like; specific examples of these include tris-(hydroxymethyl)aminomethane salts ("Tris buffer"), and disodium edetate.

In one embodiment of the invention, IgE antagonist formulations comprise a buffer, a salt, optionally, a polyol, and optionally, a preservative.

One exemplary formulation of the invention is a liquid formulation of about 1-100 mg/ml IgE antagonist in 10 mM acetate buffer, pH 5.0-6.5, 100-200 mM sodium chloride, and about 0.01% polysorbate 20, more preferably about 5 mg/ml IgE antagonist in 10 mM acetate buffer, pH 5.2, 142 mM sodium chloride, and 0.01% polysorbate 20. In other embodiments of the invention, the formulation may be freeze-dried and reconstituted for administration. For example, anti-IgE antibody can be formulated at about 25 mg/ml in 5 mM histidine, pH 6.0, and 88 mM sucrose, freeze-dried, and reconstituted in water to 100 mg/ml antibody for administration. Mixed sugars can also be used, such as a combination of sucrose and mannitol, etc.

In general, unless otherwise specified, the abbreviations used for the designation of amino acids and the protective groups used therefor are based on recommendations of the IUPAC-IUB Commission of Biochemical Nomenclature (Biochemistry, 11:1726-1732 (1972). The nomenclature used to define compounds of the invention is that specified by the IUPAC, published in European Journal of Biochemistry 138:9-37 (1984).

Therapy of interstitial cystitis can be combined with other known therapies for allergy and/or interstitial cystitis, including corticosteroids, immunosuppressive drugs, anti-inflammatory drugs, antihistamines, pentosanpolyphosphate, heparin, amitriptyline, dimethyl sulfoxide, oxychlorosene sodium, silver nitrate, disodium chromoglycate, etc.

Further details of the invention can be found in the following examples, which further define the scope of the invention. All references cited herein are expressly incorporated by reference in their entireties.

EXPERIMENTAL RESULTS

In this study a model of passively sensitized human and monkey bladder tissues was used to test the therapeutic potential of an IgE antagonist for the treatment of interstitial cystitis.

Human bladder segments were obtained from cystectomies performed to treat bladder carcinoma. Only macroscopically healthy portions of the bladder were used. However, all patients were receiving medical therapy to treat the carcinoma at the time tissues were obtained. In contrast, monkey bladder tissue was obtained from healthy animals euthanized for research purposes.

Bladder tissues were passively sensitized using the method of Bjorling et al. (J. Urol. 152:1603, 1994). Basically, bladder tissue was cleansed and prepared as entire (neck to dome) full thickness strips (5 X 18 mm). Tissue segments were placed in physiologic salt solutions (PSS) of the following composition: 119 mM NaCl/ 4.7 mM KCl/ 1.0 mM NaH_2PO_4/ 0.5 mM $MgCl_2$/ 2.5 mM $CaCl_2$/ 25 mM $NaHCO_3$/ 11 mM glucose at pH 7.4, gassed with 95% O_2 and 5% CO_2, and maintained at 37°C. Bladder segments were washed four times with 50 ml PSS, placed in 50 ml of PSS, and passively sensitized by incubation for 15 to 20 hr at 25°C with a 1:10 dilution of serum from a ragweed-allergic patient. The total IgE content of the serum was 1250 ng/ml, as determined by ELISA.

To test the ability of an IgE antagonist to block sensitization, tissues were sensitized in the presence of anti-IgE antibody E25 (Presta et al., supra) at concentrations 1, 5, and 10 times the amount of serum IgE or with control IgG at 10 times the concentration of serum IgE. Tissues incubated with PSS only were used as negative controls, and those incubated with ragweed serum only were preserved as positive controls.

After sensitization, each tissue was suspended in an air-filled tissue chamber and superfused with PSS. All solutions, as well as the tissue chamber, were maintained at 37°C. PSS was pumped from a reservoir through Tygon tubing to a water-jacketed coiled glass tube heat exchanger using a Gilson Minipulse II peristaltic pump. To remove excess sera, tissues were allowed to equilibrate for 90 minutes while perfused with PSS (1 ml/min) and maintained at a tension of 1.5 g. Changes in tension were recorded via force displacement transducers (FT-03, Grass Instruments, Quincy, MA) on a polygraph (Model 7D, Grass Instruments, Quincy, MA). Following equilibration, tissues were superfused (1 ml/min) with ragweed antigen (AgE) (0.01 to 3 µg/ml) diluted in PSS. Superfusate samples were collected at 60 second intervals, beginning one minute before and continuing 16 minutes during challenge with IgE. Contractile responses were calculated as a percent of maximal contraction induced by KCl (200 mM) added at the end of each experiment. After collection, superfusate samples were placed on crushed ice for subsequent analysis of histamine content. The remaining tissue histamine was extracted with 0.4 N perchloric acid.

To test the safety of the anti-IgE monoclonal antibody E25, adjacent segments of human bladder were passively sensitized with human ragweed serum. Following sensitization, bladder segments were suspended in tissue baths containing 10 ml of PSS (37°C) and maintained at a tension of 1.5 g for 1 hr of equilibration during which the PSS was changed every 15 minutes. Changes in tension were recorded via force displacement transducers (FT-03) on a Grass polygraph (Model 7D). After the equilibration period, tissues were challenged for 30 min with E25 (10 µg/ml) or AgE (1 µg/ml), the latter used as a positive control.

Histamine content of the superfusate and tissues was analyzed by an automated fluorometric method with a sensitivity of 1.5 ng/ml. The net concentration of histamine (release minus spontaneous) in each superfusate sample was expressed as a per cent of total histamine in each tissue prior to collection.

The mean and SEM were calculated for all data. Means were compared by analysis of variance or Student's t test. A value of $p < 0.05$ was considered indicative of significant difference.

When passively sensitized segments of human bladder were challenged in vitro with different AgE concentrations added in a cumulative fashion to the tissue bath, a concentration-dependent contraction was observed (Figure 1). Cumulative responses to AgE were maximal at 1 μg/ml and decreased when tissues were exposed to 3 μg/ml. To verify if the decreased response to high concentration of antigen was due to a desensitization phenomena, individual tissue segments were incubated with single concentrations of antigen and responses were observed over time (Figure 2). The responses were maximal for 1 μg/ml antigen. Indeed, contraction in response to 3 μg/ml AgE was lower than concentration induced by 1 μg/ml. The responses of monkey bladder were similar (data not shown). Therefore, a concentration of 1 μg/ml AgE was used in all subsequent experiments as described below.

Figure 3 illustrates representative contractile responses typically observed with antigen challenge of tissues obtained from eight different human bladders utilized in this experiment. The tissue segment in Figure 3A was not sensitized and served as a negative control. Adjacent segments of the same human bladder (3B, C, and D) were passively sensitized in vitro. AgE (1 μg/ml) challenge failed to induce contraction of the non-sensitized bladder segment (Figure 3A), which exhibited only spontaneous motility. The contractile responses to AgE (1 μg/ml) were evident when a bladder tissue segment was used as a positive control for sensitization. Tissue segments treated with equimolar E25 (1:1 IgE to E25 concentrations) during sensitization had both a reduced and delayed response to AgE (Figure 3C). Tissue segments treated with E25 (5-fold excess E25) during sensitization exhibited no measurable response to AgE (Figure 3D). All tissue segments contracted in response to KCl (200 mM) added at the end of experiments, confirming viability.

Figure 4 summarizes results of histamine release obtained with human urinary bladder segments passively sensitized in the presence of E25. E25 blocked AgE-induced histamine release and tissue contraction in a concentration-dependent manner. Equimolar concentrations of serum IgE and E25 resulted in significant inhibition of both histamine release and tissue contraction. Five-fold excess concentration of E25 completely blocked the response to AgE challenge. In contrast, tissues preincubated with ragweed serum and a 10-fold excess concentration of control IgG had histamine release and tissue contraction indistinguishable from the positive control tissues. Similar results were observed with the Rhesus monkey bladder tissue (Figure 5 A-B). There was a dose-dependent inhibition of sensitization and a five-fold molar excess of E25 was need to completely block response to IgE challenge.

Challenge of either passively sensitized human or monkey bladder tissue with AgE at 1 μg/ml induced histamine release and tissue contraction (Figure 6). However, in striking contrast, no histamine release or tissue contraction was observed in response to challenge with up to 10 μg/ml E25. These results indicate that E25 does not bind human IgE that is already bound to high affinity receptors on human and monkey bladder mast cells.

Thus, in these experiments, an IgE antagonist effectively blocked mast cell degranulation and histamine release in a model for non-infectious cystitis. These results indicate that an IgE antagonist can be used therapeutically in the treatment of interstitial cystitis.

WE CLAIM:

1. A method of treatment of interstitial cystitis in a patient comprising administering a therapeutic dose of an IgE antagonist to the patient.

2. The method of claim 1, wherein the therapeutic dose comprises a maintenance dose of an IgE antagonist and, optionally, a loading dose of the IgE antagonist.

3. The method of claim 2, wherein the maintenance dose is repeated at intervals of about 1 to about 90 days.

4. The method of claim 2, wherein the maintenance dose is repeated weekly.

5. The method of claim 2, wherein the maintenance dose is repeated biweekly.

6. The method of claim 1, wherein the IgE antagonist is an anti-IgE antibody.

7. The method of claim 6, wherein the antibody is chimeric.

8. The method of claim 7, wherein the antibody is humanized.

9. The method of claim 6, wherein the antibody is a human antibody.

10. The method of claim 2, wherein the antagonist binds to soluble IgE and blocks the binding of IgE to the IgE receptor on basophils.

11. The method of claim 6, wherein the antibody binds to soluble IgE and blocks the binding of IgE to the IgE receptor on basophils.

12. The method of claim 2, wherein the loading dose is greater than the maintenance dose.

13. The method of claim 1, wherein the therapeutic dose reduces the concentration of free IgE in the patient's serum to less than about 40 ng/ml.

14. The method of claim 1, wherein the therapeutic dose of antagonist is about 0.001 to 0.01 mg/kg/week/baseline IgE IU/ml.

15. The method of claim 1, wherein the therapeutic dose results in a total serum concentration of antagonist of about 1 to 10 times greater than the patient's total serum IgE concentration.

16. The method of claim 1, wherein the therapeutic dose of IgE antagonist averages about 0.001 to 0.01 mg/kg/week IgE antagonist for every IU/ml baseline IgE in the patient's serum.

17. A method of reducing histamine release from mast cells in the bladder tissue of a patient with interstitial cystitis comprising administering a therapeutic dose of an IgE antagonist to the patient.

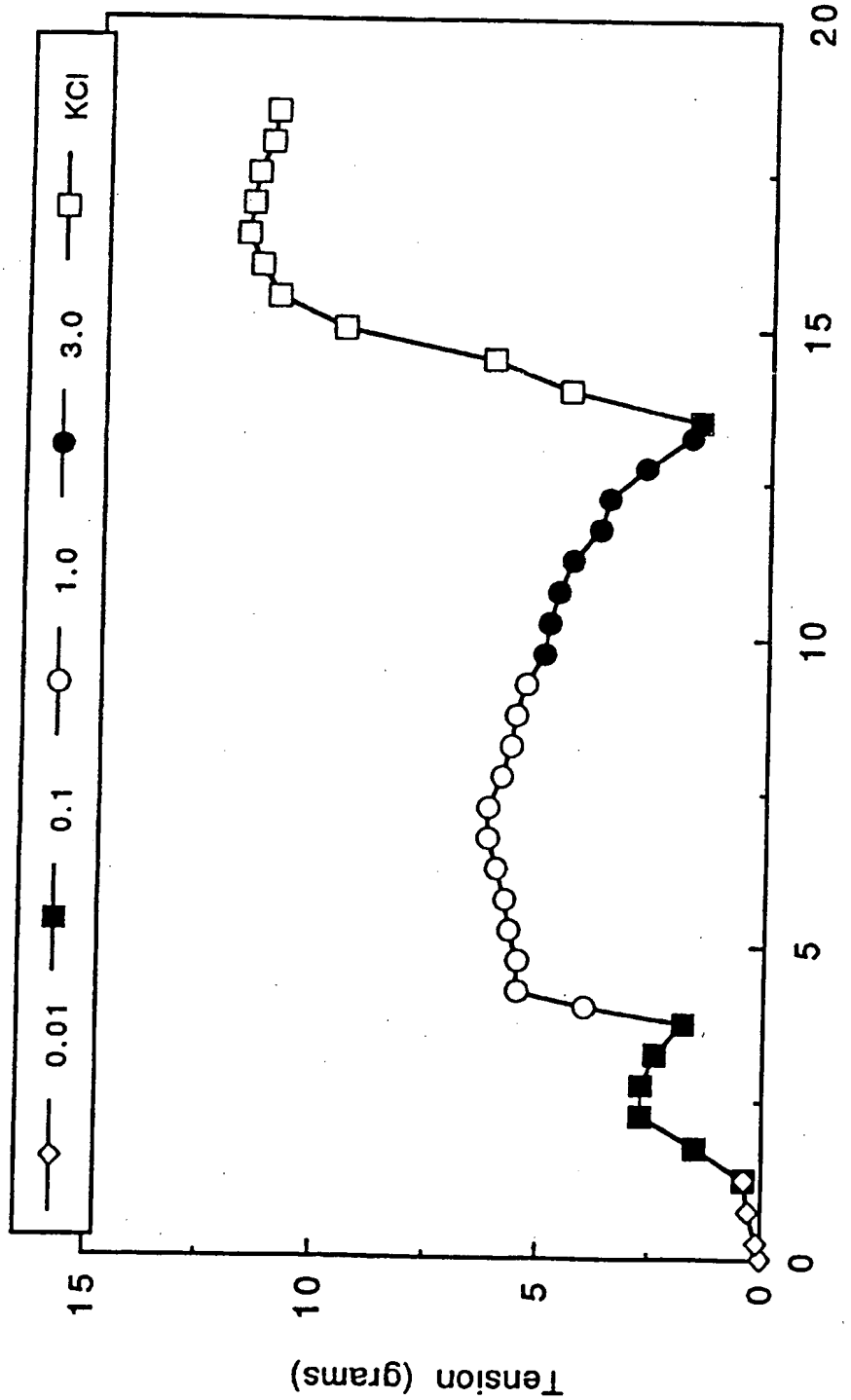


FIG. 1

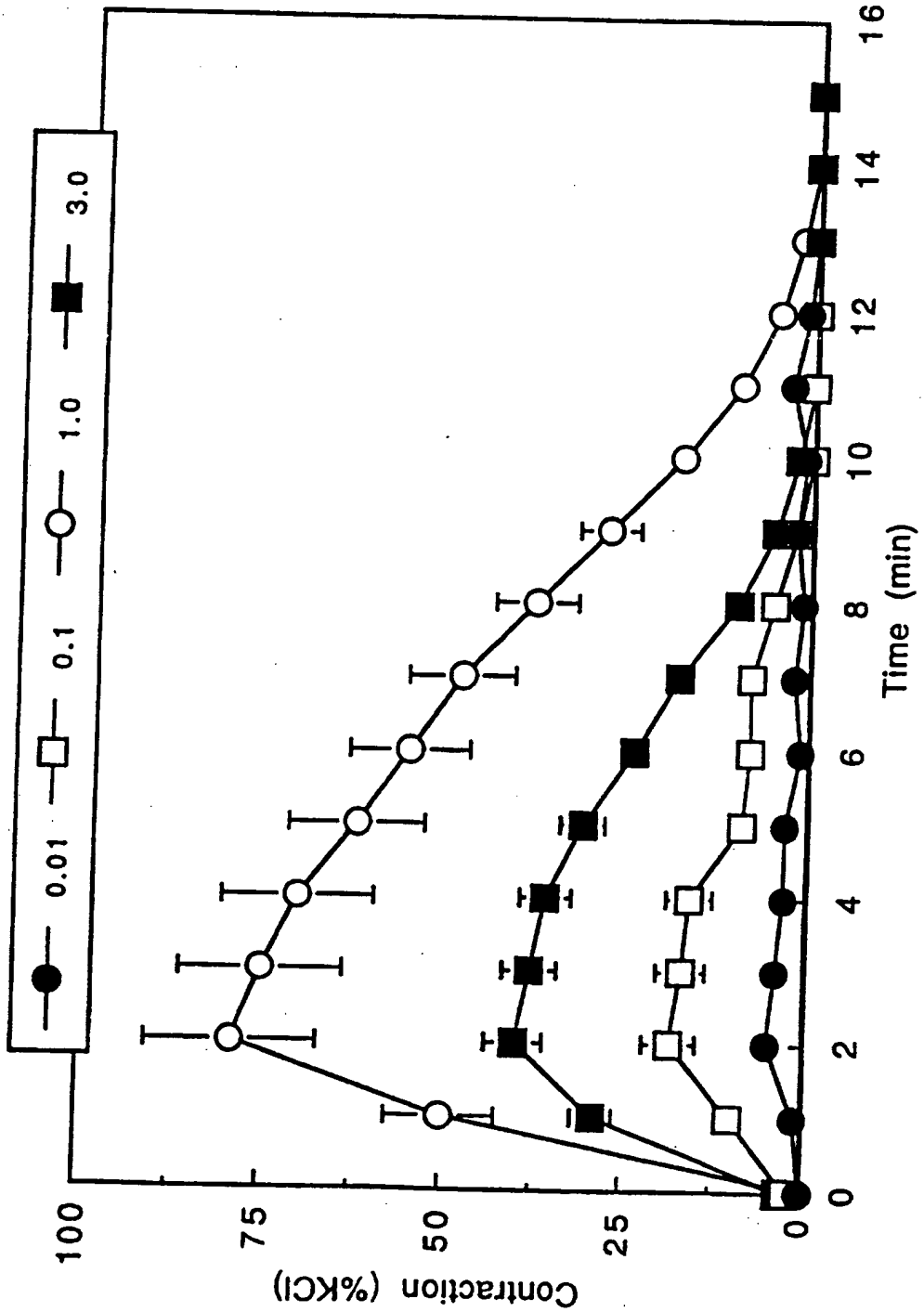

0.01
0.1
1.0
3.0
Contraction (%KCl)
100
75
50
25
0
0
2
4
6
8
10
12
14
16
Time (min)

FIG. 2

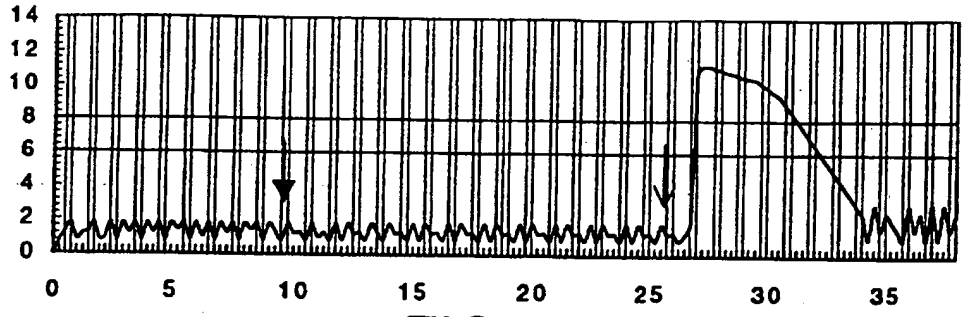


FIG. 3A

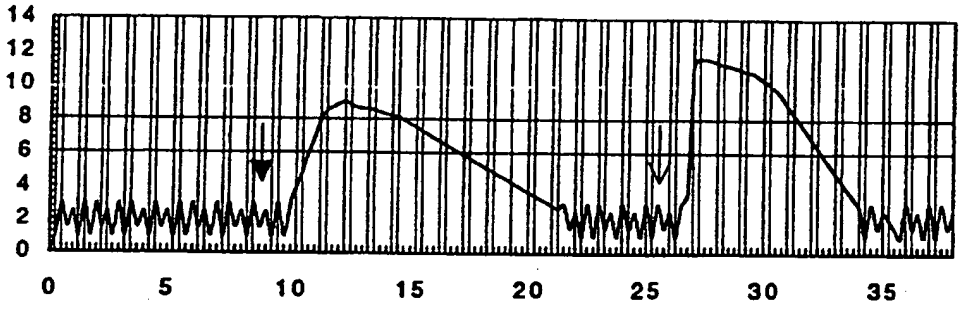


FIG. 3B

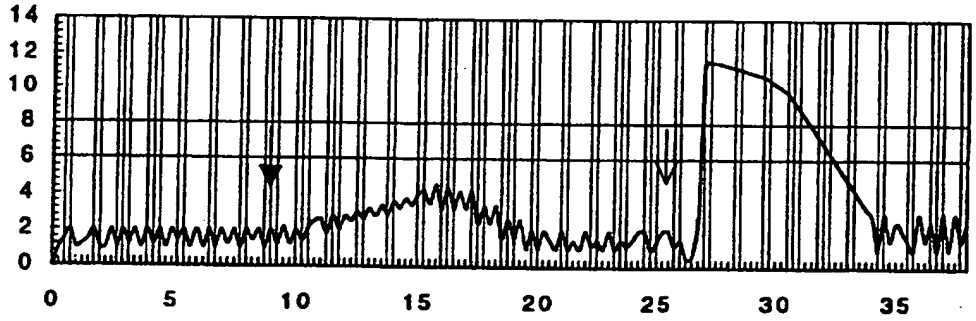


FIG. 3C

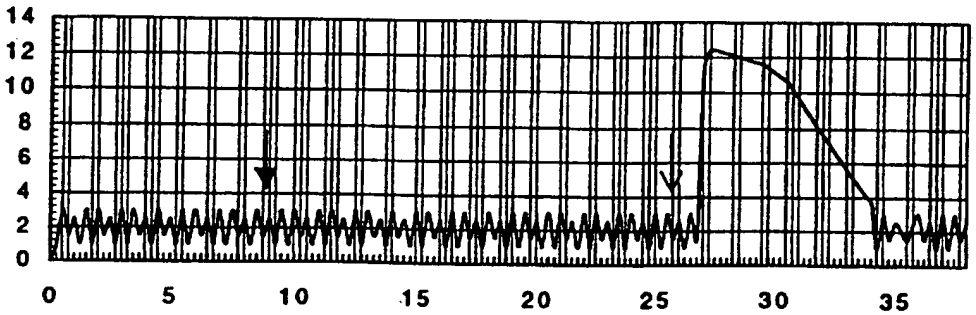


FIG. 3D

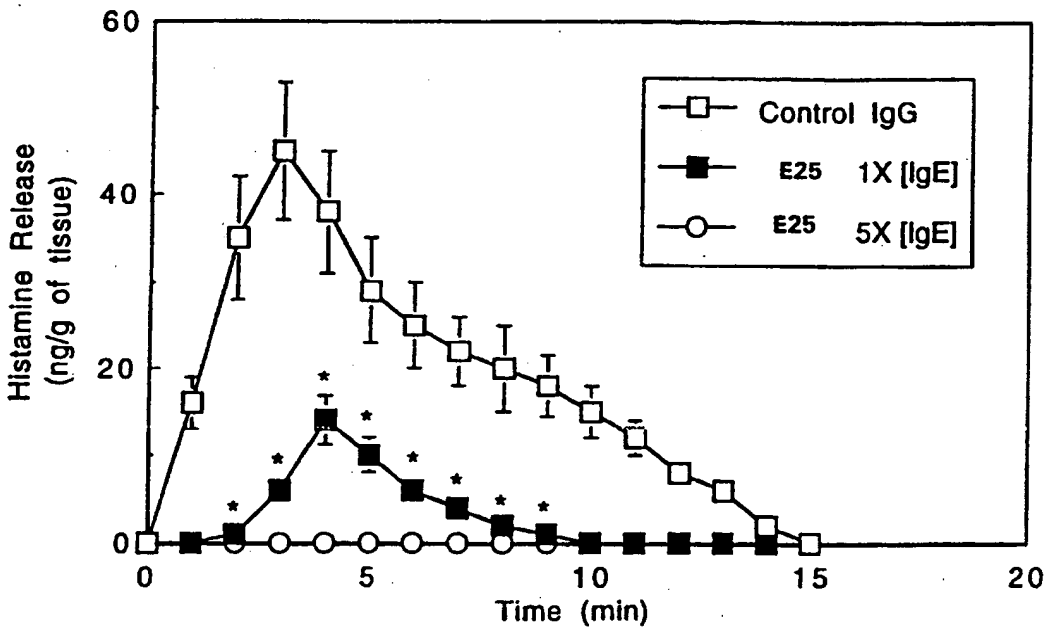

Histamine Release
(ng/g of tissue)
60
40
20
0
0
5
10
15
20
Time (min)
Control IgG
E25 1X [IgE]
E25 5X [IgE]

FIG. 4A

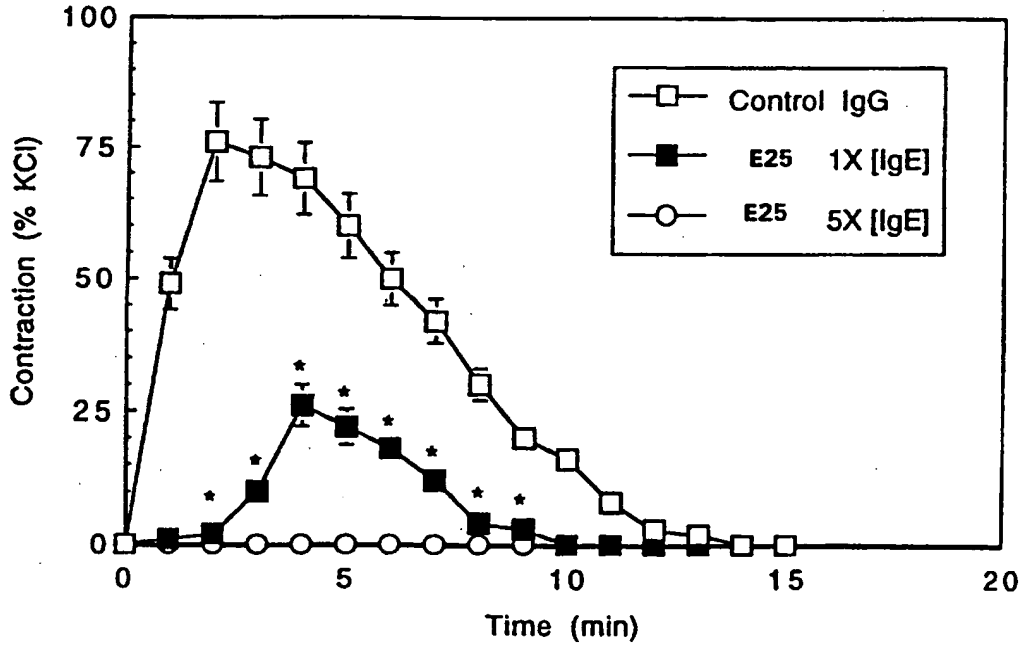

Contraction (% KCl)
100
75
50
25
0
0
5
10
15
20
Time (min)
Control IgG
E25 1X [IgE]
E25 5X [IgE]

FIG. 4B

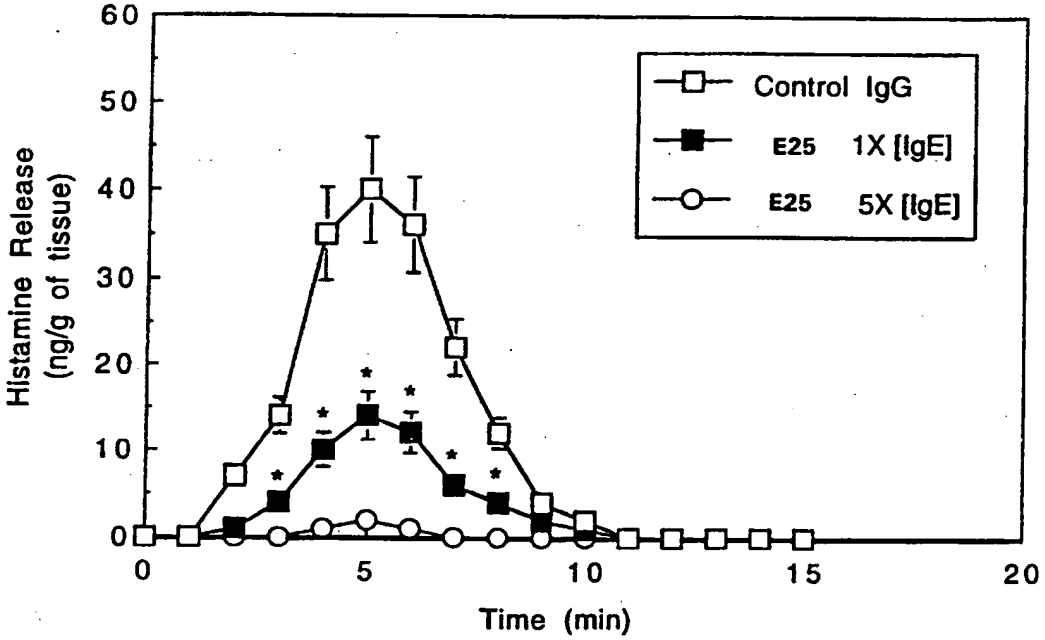
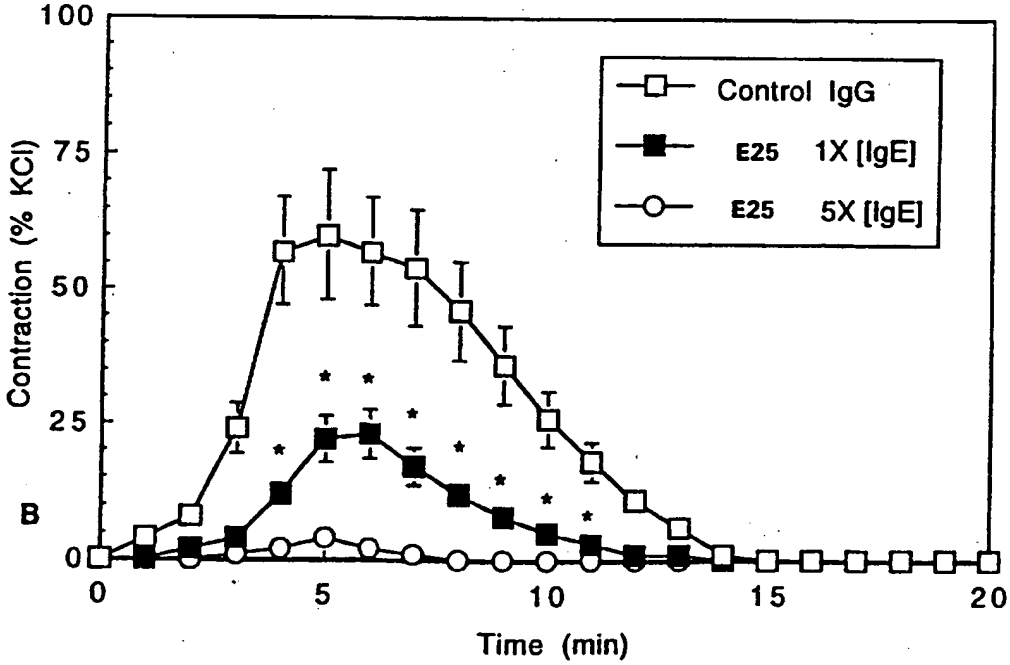


FIG. 5A



B

FIG. 5B

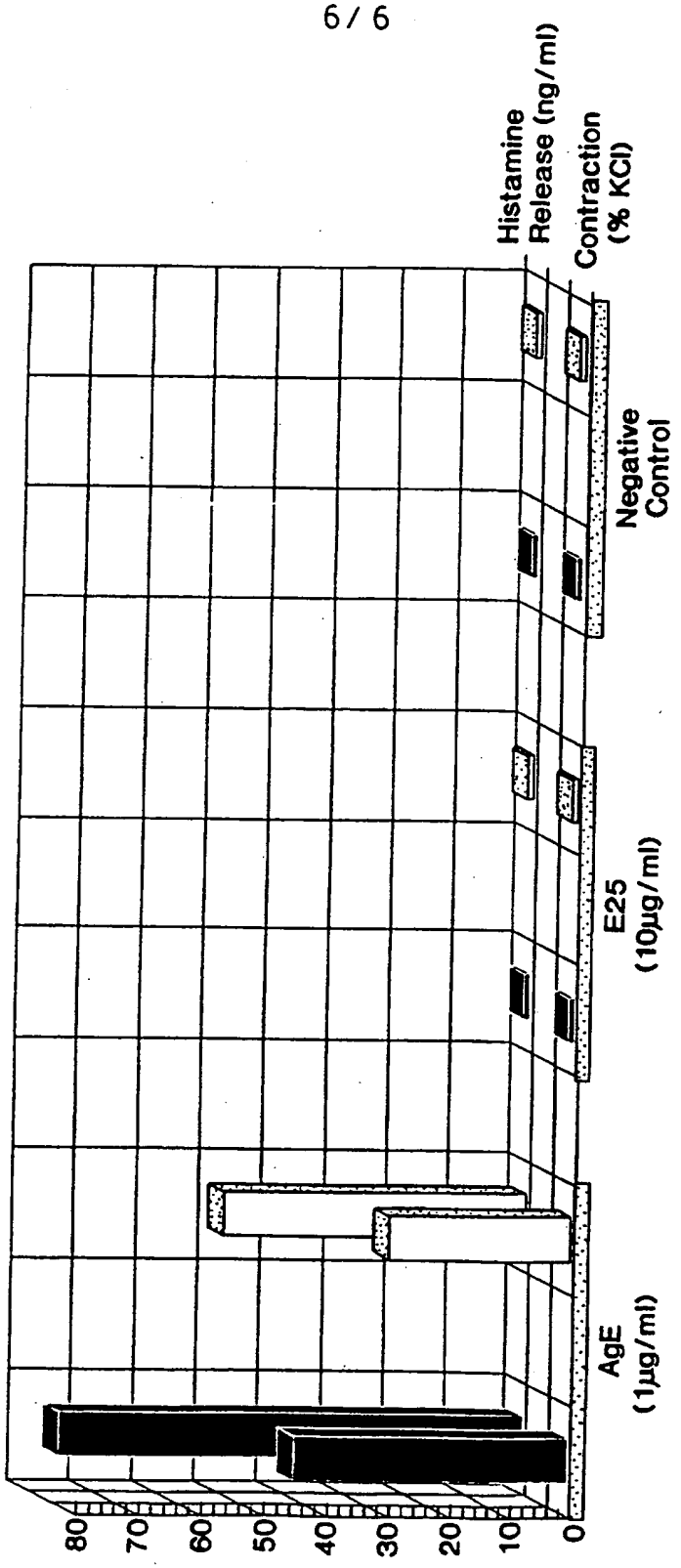

Histamine Release (ng/ml)
Contraction (% KCl)
Negative Control
E25 (10µg/ml)
AgE (1µg/ml)
80
70
60
50
40
30
20
10
0

FIG. 6

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 96/14548

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THE FASEB JOURNAL, vol. 8, no. 5, 18 March 1994, BETHESDA, MD, USA, page A682 XP002021719 R. SABAN ET AL.: "Humanized anti-IgE monoclonal antibody MaE25 blocks passive sensitization of rhesus monkey bladder in vitro." see abstract 3953 ---	1-17
Y	THE NISHINIHON JOURNAL OF UROLOGY, vol. 48, no. 5, 1986, FUKUOKA, JAPAN, pages 1487-1492, XP000615379 S. KANDA ET AL.: "Allergic cystitis immunohistological study of the localization of immunoglobulin E and etiological diagnosis." see the English summary ---	1-17

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 December 1996	10.01.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCI/US 96/14548

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 589 840 A (CIBA-GEIGY AG & TANOX BIOSYSTEMS, INC.) 30 March 1994 see examples see claims	1-17
Y	WO 92 17207 A (TANOX BIOSYSTEMS, INC.) 15 October 1992 see example see claims	1-17
Y	IMMUNOLOGY, vol. 58, no. 3, July 1986, OXFORD, GB, pages 411-416, XP000615477 F. ALDENBORG ET AL.: "Proliferation and transepithelial migration of mucosal mast cells in interstitial cystitis." see page 415, left-hand column, line 59 - right-hand column, line 18	1-17
A	THE JOURNAL OF IMMUNOLOGY, vol. 151, no. 1, 1 July 1993, BALTIMORE, MD, USA, pages 351-358, XP002021720 M. HAAK-FRENDSCHO ET AL.: "Human IgE receptor alpha-chain IgG chimera blocks passive cutaneous anaphylaxis reaction in vivo." cited in the application see abstract	1-5,10, 12-17
A	THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, vol. 94, no. 5, November 1994, ST. LOUIS, MO, USA, pages 836-843, XP000615476 R. SABAN ET AL.: "Human FcERI-IgG and humanized anti-IgE monoclonal antibody MaE11 block passive sensitization of human and rhesus monkey lung." see abstract see discussion	1-17
A	WO 93 19197 A (IMMUNET) 30 September 1993 see examples see claims	1-6,9-17
A	THE FASEB JOURNAL, vol. 9, no. 4, 10 March 1995, BETHESDA, MD, USA, page A1047 XP002021721 F. PEARCE ET AL.: "Changes in mast cell reactivity in the course of allergic inflammation." see abstract 6067	1-17

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 96/ 14548

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-17
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-17 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCI/US 96/14548

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-589840	30-03-94	AU-A-	4748893	31-03-94
		CA-A-	2106719	25-03-94
		CN-A-	1088986	06-07-94
		FI-A-	934145	25-03-94
		JP-A-	6225788	16-08-94
		NO-A-	933394	25-03-94
		NZ-A-	248743	27-04-95
		ZA-A-	9307033	11-08-94
WO-A-9217207	15-10-92	EP-A-	0585224	09-03-94
WO-A-9319197	30-09-93	AU-A-	3923293	21-10-93
		EP-A-	0651819	10-05-95
		JP-T-	7507923	07-09-95